MORPHOLOGICAL COLOR CHANGE IN THE HAWAIIAN GHOST CRAB, OCYPODE CERATOPHTHALMA (PALLAS)[1, 2, 3]

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The functional activities of crustacean chromatophores are manifested by two types of color change. The relatively rapid mobilization of the pigment, leading to its concentration or dispersal within the chromatophore, is referred to as physiological color change. This facet of chromatophore activity is contrasted with morphological color change, a more enduring modification resulting from the production or destruction of pigment and/or chromatophores.

The relation between the physiological color change, as mediated by various hormonal agents, and the mechanisms controlling morphological color changes is imperfectly understood. Experimental studies in the past have been concerned principally with the hormonal control of the transitory physiological color changes (see reviews by Brown, 1961, and Fingerman, 1963). Few investigators have given any attention to the formation or destruction of crustacean pigments as a result of prolonged stimulation.

Sumner (1940) credits Flemming (1882) with the first observation that background shade could influence the total color pattern of an animal. Typical background responses result in dispersion of dark pigments on dark backgrounds and concentration on light backgrounds. Light colored chromatophores respond in an opposite manner. Babak (1912) postulated a relationship between physiological and morphological color changes, based on the state of dispersion of pigments within the chromatophore. This relationship, subsequently called Babak's Law, stated that the maintenance of a pigment in a concentrated state within the chromatophore is correlated with a reduction in the quantity of that pigment. Conversely, pigment dispersion is associated with pigment production.

Morphological color changes have been suggested to occur in the Crustacea (Keeble and Gamble, 1904) but only two papers have offered any experimental evidence for this phenomenon. Brown (1934) investigated the morphological color changes of the prawn, *Palaemonetes vulgaris*, maintained on various backgrounds. He observed that the red and blue pigments were the most rapidly formed

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and destroyed, while the yellow and white pigments were less reactive when the prawns were maintained on the appropriate background. Brown (p. 379) concludes that ". . . it is possible that pigment formation and destruction in adaptation to colored backgrounds may be controlled by the same humoral agents that are responsible for the control of migration of the pigments within the chromatophores."

Bowman (1942) counted crayfish (*Cambarus clarkii*) chromatophores and found that after 52 days on a black background the number of white chromatophores decreased as compared to the original number and that the number of red chromatophores increased. On a white background the converse was true.

Morphological color changes can be accomplished by more than one process: (1) by an increase (decrease) in the number of chromatophores per unit area of body surface (Bowman, 1942); (2) by an increase (decrease) in pigment concentration (Brown, 1934; Green, 1963); or (3) by a combination of the above.

The present study deals with the capacity of the Hawaiian ghost crab, *Ocypode ceratophthalma* (Pallas) to undergo morphological color change.

Materials and Methods

Male ghost crabs were collected periodically from four different areas on the islands of Oahu and Hawaii, Hawaii. The beaches on Oahu are composed of finely divided carboniferous, diatomaceous, and foraminiferous sand, and present a white background. In contrast to the Oahu beaches, the beach on Hawaii is composed of lava sand and presents a black background. The crabs from both beaches merit the name "ghost crab" because of their ability to blend with the background and their near invisibility when in motion.

Crabs were collected as needed on the Oahu beaches and placed in a bucket with sand. The time elapsed between collection and subsequent return to the laboratory seldom exceeded one hour. Crabs from the island of Hawaii were collected on a Thursday afternoon and placed by twos in wax-coated paper cups with black sand on the bottom and maintained in these containers until the following Tuesday afternoon.

Upon reaching the laboratory the crabs were washed free of adhering sand and the number of black chromatophores on the anterior surface of the meropodite of the fourth right walking leg counted with the aid of a Bausch and Lomb dissecting microscope (× 13). After the chromatophores were counted the approximate area of the anterior surface of the meropodite was determined by direct measurement. Analysis of the number of black chromatophores per unit area of the legs of the crabs from the three Oahu beaches employed as collection sites indicated that the crabs were comparable with respect to this parameter.

The animals were then placed on one of four regimes:

A. White background—constant illumination
B. Black background—constant illumination
C. Total darkness
D. White background—intermittent illumination (12 hours light, 12 hours dark).

The animals were kept in individual glass vessels three inches high and three inches in diameter, painted either white or black on the outside. One-fourth inch

TABLE I

Effect of various regimes on the black chromatophore index

Collection location	Number of animals	Treatment	Time on treatment (days)	Black chromatophore index Mean ± S.E.
White sand beaches	160	—	0	3.14±0.10
White sand beaches	17	White background, constant illumination	7	4.60±0.65
White sand beaches	21	White background, constant illumination	14	5.15±0.71
White sand beaches	8	White background, constant illumination	21	5.18±0.76
White sand beaches	3	White background, constant illumination	28	5.48±2.09
White sand beaches	18	Black background constant illumination	7	5.32±0.49
White sand beaches	9	Black background, constant illumination	14	8.67±1.23
White sand beaches	4	Black background, constant illumination	21	6.35±1.34
White sand beaches	6	Total darkness	7	3.01±0.32
White sand beaches	5	Total darkness	14	3.13±0.30
White sand beaches	5	White background, intermittent illumination	7	3.07±0.58
White sand beaches	5	White background, intermittent illumination	14	2.71±0.51
Black sand beach	24	—	0	37.22±0.70
Black sand beach	5	White background, constant illumination	7	32.66±2.03

sea water was kept in each vessel and fresh sea water was added every two or three days. The animals were unfed during the experimental period (which amounted to at most four weeks for any one animal). Blanks in the data are due to death of the experimental animal. The animals were illuminated by General Electric "daylight" fluorescent light bulbs. The animals maintained in total darkness were kept in a photographic darkroom and were illuminated by a Nicholas illuminator only for the time necessary to count their chromatophores (about one minute). After 7, 14, 21, and 28 days had elapsed the numbers of black chromatophores on the same area of the leg were recounted. In order to facilitate counting chromatophores with dispersed pigment, *i.e.*, from animals from the black sand beach or those on black

TABLE II

The relationship between black chromatophore number and the area of the anterior surface of the meropodite of the fourth right walking leg of newly caught Ocypode ceratophthalma

Collection location	Number of animals	Number of black chromatophores		Leg area (mm.²)	
		Range	Mean ± S.E.	Range	Mean ± S.E.
White sand beaches	160	11–261	70 ± 4	6.0–51.5	21.0 ± 0.87
		Black chromatophore index = 3.14 ± 0.01			
Black sand beach	24	250–630	425 ± 23.5	6.4–16.8	11.4 ± 0.12
		Black chromatophore index = 37.22 ± 0.70			

backgrounds, the expanded chromatophores were made to contract by placing the animals in white bowls for two hours.

Results

The data are presented in Table I. The means listed in the table are those of the black chromatophore number per unit area (mm.^{2}) of the anterior surface of the meropodite of the fourth right walking leg (black chromatophore index) ± the standard error of the mean. The relationship between black chromatophore number and the area of the meropodite of newly caught animals is presented in Table II.

Regression analyses have been obtained for the animals maintained on the various regimes. In all cases the black chromatophore index has been related to the length of time that the animals were maintained on the various backgrounds.

Figure 1A indicates that white sand animals maintained on white background with constant illumination did not show a significant regression between black chromatophore index and time of maintenance on the background. Further analysis reveals, however, that this curve contains a component which indicates a significant increase in black chromatophore number during the first seven days on the white background. Thereafter (7 through 28 days) no further significant change occurs.

Figure 1B indicates that there has been a highly significant increase in black chromatophore index (+ 0.3 black chromatophore per mm.^{2} per day) in white sand animals maintained on a black background. This increase is considered to be linear over the experimental period. The decrease observed between the 14th and 21st days is not statistically significant. The high variability of the mean for the 21st day is in part due to the small number of surviving animals.

Animals maintained in total darkness (Fig. 1C) and under intermittent illumination—white background (Fig. 1D) showed no significant variation in black chromatophore number during the experimental period.

The data for the black sand animals on white background have not been plotted because only two points are available (0 and 7 days). However, a comparison between the mean black chromatophore index for these two points indicates that there has been a significant decrease in black chromatophore index (− 0.76 black chromatophore per mm.^{2} per day) over the experimental period.

During the experimental period the black chromatophores of those black sand animals kept on a white background underwent certain structural changes. The pigment within the black chromatophores contracted and the pigment granules became densely packed together at the cell center. Due to the large number of black chromatophores and their syncytial nature in the hypodermis lining the carapace, the animals did not immediately appear markedly lighter. Several days later, after being on the white background under constant illumination, some of the black chromatophores appeared to be degenerating. Dark-colored particles could be seen scattered among the intact chromatophores. In general, the freed pigment particles tended to form a corona about the old cell center. The processes of the black chromatophores of white sand animals kept on a black background showed increased arborization and processes of adjoining "expanded" chromatophores intermingled, thereby temporarily losing their identity.

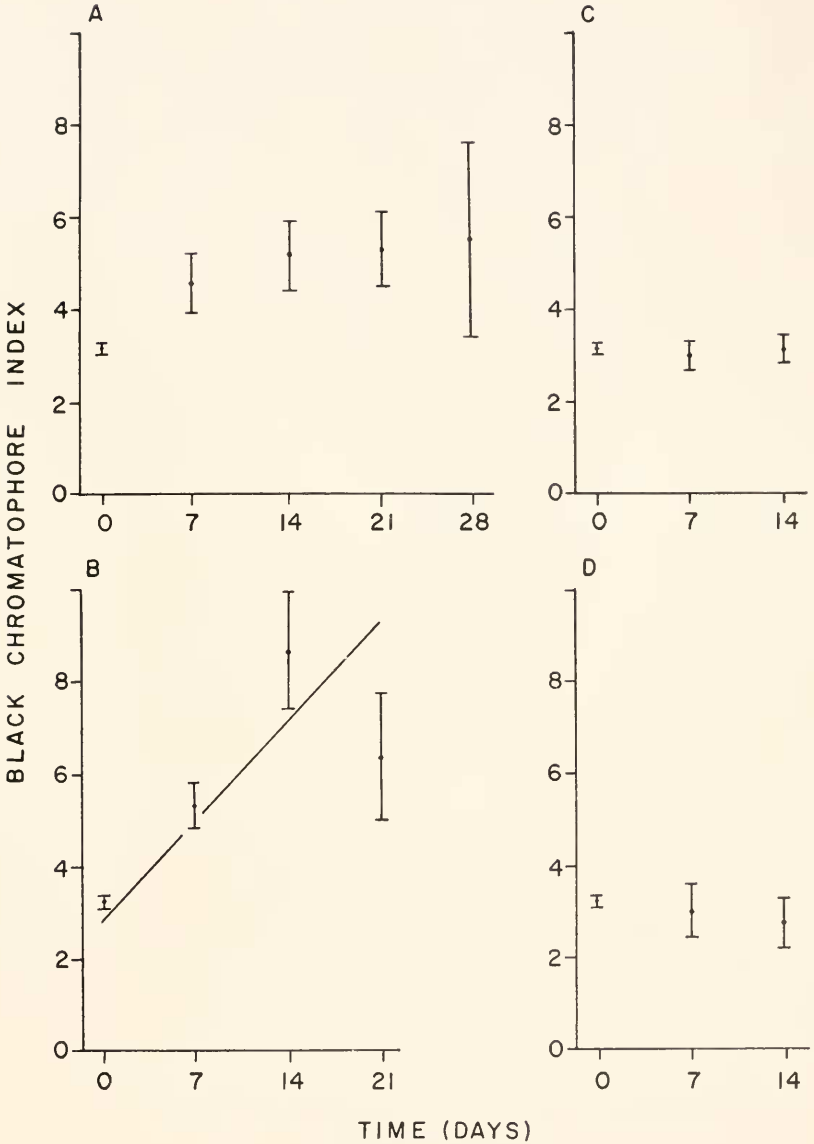

FIGURE 1. The relationship between black chromatophore index and time spent on background (for crabs of white sand history). The $P = 0.95$ confidence interval of the slope of the linear regression line is given. A. White background—constant illumination. $-0.005 \leqslant b \leqslant +0.249$. B. Black background—constant illumination. $+0.243 \leqslant b \leqslant +0.353$. C. Total darkness. $-0.110 \leqslant b \leqslant +0.050$. D. White background—intermittent illumination. $-0.075 \leqslant b \leqslant +0.067$.

Discussion

The two populations of Hawaiian ghost crab, one from the white sand beaches of Oahu and the other from the black sand beach of Hawaii, are (aside from pigmentation) morphologically indistinguishable. The black sand crab has, however, nearly 12 times as many black chromatophores as does its white sand counterpart.

Long-term maintenance of crabs on particular backgrounds in some cases effects changes in the number of black chromatophores. The most interesting cases are those of white sand animals on black background and of black sand animals on white background. In the first case there has been a highly significant increase in the black chromatophore index and in the second case a highly significant decrease. This finding can be contrasted with crabs maintained in total darkness or under conditions of intermittent illumination (white background). Crabs in the latter two regimes did not show any significant variation in black chromatophore index over the same time period. The black chromatophores of white sand animals on white background increased during the first seven days on white background but thereafter remained at this new level for the remainder of the experimental period.

I am unable to explain the increase in the black chromatophore index of animals of white sand history maintained on a white background, but it is noteworthy that this increase persisted only for the first seven days and no further increase thereafter occurred. This group of crabs was under unnatural light conditions and therefore it is not particularly surprising to find differences between animals continuously illuminated and those which were intermittently illuminated.

We can conclude from these results that, in addition to other less well-known factors, illumination and background shade influence formation and destruction of chromatophores in *Ocypode*.

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Summary

1. The black chromatophores of two populations of the Hawaiian ghost crab, *Ocypode ceratophthalma* (Pallas), were investigated with respect to the effect of long-term background adaptation.

2. Crabs from the black sand beach of Hawaii have twelve times the number of black chromatophores as do crabs from the white sand beaches of Oahu.

3. Crabs of white sand history maintained on a black background in the laboratory showed an increase of 0.3 black chromatophore per mm.^{2} per day over a 28-day period.

4. Crabs of black sand history maintained on a white background in the laboratory showed a decrease of 0.76 black chromatophore per mm.^{2} per day over a 7-day period.

5. The formation and destruction of black chromatophores in *Ocypode* is related to factors of illumination and background shade.

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